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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c).

INVENTOR(S)				
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<input checked="" type="checkbox"/> Additional inventors are being named on the <u>attached</u> separately numbered sheets attached hereto				
TITLE OF THE INVENTION (280 characters max)				
METHODS FOR PREVENTING NEUROLOGICAL EVENTS				
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ENCLOSED APPLICATION PARTS (check all that apply)				
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METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT (check one)				
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Respectfully submitted,
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Date 8/12/2003

REGISTRATION NO. 27,969
(if appropriate)

Docket Number: GLYCO-26V1

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PROVISIONAL APPLICATION COVER SHEET
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Title: Methods for Preventing Neurological Events

FIELD OF THE INVENTION

The invention relates generally to preventing or reducing neurological events prior to, during, or following medical or surgical procedures.

5 **BACKGROUND OF THE INVENTION**

There have been important advances in cardiac surgery in the last decades including procedures such as coronary artery bypass grafting (CABG) and cardiac repair or replacement surgery. Cardiopulmonary bypass (CPB) is generally used in these procedures to divert blood through an extracorporeal circuit to allow for the
10 patient's heart and lungs to be stilled during surgery.

There has been growing awareness of the adverse neurological effects associated with cardiac surgery, and in particular CPB. Approximately 500,000 people each year in the United States alone undergo cardiovascular surgical procedures that use cardiopulmonary bypass (CPB) and neurocognitive deficits have been reported to
15 occur in over 50% of these patients (Newman et al. "Longitudinal Assessment of Neurocognitive Function After Coronary-Artery Bypass Surgery," New England Journal of Medicine 2001; 344: 395-402). The reported incidence, as measured by neuropsychological testing, ranges from 40 – 61% within the first week following surgery (Gugino LD et al, 1999; Vingerhoets, G. et al, 1997; Rodig G. et al, 1999;
20 Newman MF, et al, 2001; Grigore AM et al, 2002; and Llinase R et al, 2000). Although there is substantial resolution in neurocognitive dysfunction within 6 weeks to 6 months, up to 35% of patients have neurocognitive defects that persist for at least a year (Di Carlo, A et al, 2001). Neurocognitive deficits also occur with other forms of surgery (Vingerhoets, G, et al 1997; Van Dijk, D. et al, 2002), but the incidence of
25 neurocognitive deficits is highest after CPB.

The etiology of neurocognitive dysfunction is thought to be the result of microemboli. Several studies have been performed using Transcranial Doppler to detect microemboli as high-intensity transient signals (HITS) during cardiac surgery

(Di Carlo A et al, 2001 and Jacobs, A. et al, 1998). In these studies, HITS were associated with neurocognitive deficits, especially with respect to memory loss. Possible sources of the microemboli include air, thrombi, and fat from cellular or particulate matter promoted by the bypass pump (Jacobs A et al, 1998). Of these, 5 thromboemboli are thought to be most important.

Currently, unfractionated heparin (UFH) is the standard agent used for anticoagulation during cardiopulmonary bypass (CPB). Although UFH provides sufficient anticoagulation to prevent clotting within the bypass circuit its inability to inactivate fibrin-bound thrombin (Hogg PJ and Jackson Cm, 1990 and Weitz JI et al, 10 1998) as well as its tendency to activate platelets (Xiao Z et al, 1998) limits the ability of UFH to reduce thrombin generation and the development of thromboemboli during CPB.

It would be beneficial to eliminate or reduce the potential of neurological events associated with medical and surgical procedures. In particular, there is a need 15 for substances that reduce or eliminate cardiac embolization.

The citation of any reference herein is not an admission that such reference is available as prior art to the instant invention.

SUMMARY OF THE INVENTION

The present invention relates to therapeutic methods for preventing or reducing 20 neurological events utilizing a glycosaminoglycan and a serpin. Methods of the present invention may be advantageous for protecting or reducing neurological events prior to or during medical or surgical procedures, and after a neurological event.

In particular, the present invention deals with neurological events associated with the generation of emboli (in particular thromboemboli) that can lodge in the brain 25 and/or cerebral circulation (i.e. cardiac embolization) during cardiac surgery. Neurological events such as embolization contribute to problems including stroke, lengthy hospital stays, and in some instances death.

An aspect of the invention relates to a therapeutic application of a

glycosaminoglycan and a serpin, in particular conjugates or complexes thereof, to provide protection to a subject against neurological events, or reduce such neurological events.

5 In an aspect the invention provides a method of preventing or reducing neurological events in a subject comprising administering a therapeutically effective dosage of a glycosaminoglycan and a serpin, in particular conjugates or complexes thereof, to the subject to prevent or reduce the neurological events.

10 In another aspect, the present invention relates to a therapeutic application of a glycosaminoglycan and a serpin, in particular conjugates or complexes thereof, to provide protection to an individual's central nervous system, in particular an individual's brain, prior to scheduled, or unscheduled, procedures that may affect the central nervous system, in particular, the brain.

15 In an embodiment, the invention provides a method for reducing emboli (in particular, thromboemboli) in the cerebral circulation in a subject comprising administering an amount of a glycosaminoglycan and a serpin, in particular conjugates or complexes thereof, effective to reduce the emboli.

20 The invention also relates to a method of cerebral embolic protection in a subject comprising administering an amount of a glycosaminoglycan and a serpin, in particular conjugates or complexes thereof, to prevent or reduce emboli in the cerebral circulation.

The invention relates to a method for protecting a subject against cerebral embolization comprising administering an amount of a glycosaminoglycan and a serpin, in particular conjugates or complexes thereof, that prevents or reduces the amount of emboli that reach the cerebral vasculature.

25 The invention also provides methods for eliminating or minimizing cerebral embolization during invasive cardiac procedures.

Further, the invention provides a method of preventing or reducing emboli from a bypassed heart region prior to removal of the region from bypass comprising

administering an amount of a glycosaminoglycan and a serpin, in particular conjugates or complexes thereof, to prevent or reduce the emboli.

The invention also provides a method for improving the outcome of cardiac surgery in a subject undergoing cardiopulmonary bypass surgery comprising
5 administering a therapeutically effective amount of a glycosaminoglycan and a serpin, in particular conjugates or complexes thereof.

The invention also provides a method of performing cardiac surgery, in particular, CABG surgery, in which a therapeutically effective amount of a glycosaminoglycan and a serpin, in particular conjugates or complexes thereof, are
10 administered peri-operatively to a subject undergoing cardiopulmonary bypass to reduce the effects of emboli.

In an aspect of the invention the glycosaminoglycan and serpin provide synergistic activity in preventing or reducing neurological events. In another aspect a method of preventing or reducing cerebral emboli in a subject is provided comprising
15 administering to a subject in need thereof, synergistically effective amounts of a glycosaminoglycan and a serpin.

The present invention also provides compositions comprising a combination of a therapeutically effective amount of a glycosaminoglycan and a serpin together with a pharmaceutically acceptable excipient, carrier, or vehicle. The present invention also
20 contemplates a pharmaceutical composition in separate containers and intended for simultaneous or sequential administration, comprising a glycosaminoglycan and a serpin, both together with pharmaceutically acceptable excipients, carriers, or vehicles.

In accordance with one aspect, a pharmaceutical composition is provided comprising a glycosaminoglycan and a serpin effective to exert a synergistic effect to
25 prevent or reduce neurological events, in particular a neurological event associated with emboli more particularly, cerebral embolization. The method also provides pharmaceutical compositions comprising a synergistically effective amount of a combination of a glycosaminoglycan and a serpin in a pharmaceutically acceptable excipient, carrier, or vehicle.

In another aspect the invention relates to a method of using a glycosaminoglycan and a serpin, in particular conjugates or complexes thereof, in the preparation of a medicament to prevent or reduce neurological events, in particular neurological events associated with emboli, more particularly cerebral embolization.

5 In another aspect the invention relates to a method of using synergistically effective amounts of a glycosaminoglycan and a serpin in the preparation of a pharmaceutical composition for preventing or reducing neurological events, in particular neurological events associated with emboli, more particularly cerebral embolization.

10 These and other aspects, features, and advantages of the present invention should be apparent to those skilled in the art from the following drawings and detailed description.

DESCRIPTION OF THE DRAWINGS

The invention will be better understood with reference to the drawings in
15 which:

Figure 1 shows a timeline for the pig CPB model.

Figure 2 shows a diagram of the pig CPB model.

Figure 3 are graphs of the average microemboli HITS per hour pre hypothermic CPB (3A); average microemboli HITS per hour during hypothermic CPB (3B); percent (during/pre) hypothermic CPB microemboli HITS per hour (3C); and
20 average microemboli HITS per hour post hypothermic CPB (3D).

Figure 4 is a graph showing hypothermic CPB bleeding for the identified agents expressed as ml/hour.

Figure 5 are graphs showing protein deposition on the CPB circuit measured
25 either as total protein (5A) or as hemoglobin (5B).

Figure 6 are representative photographs of the arterial side of the blood filter and the venous side cardiectomy reservoir.

Figure 7 are graphs of the anticoagulant effect of various test agents (H300, H1000, ATH(1), ATH(3), and AT(3) + H300) and the ability of protamine sulphate to reverse these effects in the pig model.

Figure 8 is a graph showing protamine reversal of various test agents (H300, H1000, ATH(1), ATH(3), and AT(3) + H300) in the pig model.

Figure 9 are graphs showing the increase of thrombin antithrombin complexes (TAT) during CPB and thereafter in the pig model.

Figure 10 are graphs showing the levels of D-dimers during CPB and thereafter in the pig model.

DETAILED DESCRIPTION OF THE INVENTION

Glossary

"Serpín" refers to a serine protease inhibitor and is exemplified by species including but not limited to antithrombin III and heparin cofactor II. The term includes a serpin derivative. "Serpín derivative" refers to a serpin that possesses a biological activity (either functional or structural) that is substantially similar to the biological activity of a serpin. The term "derivative" is intended to include "variants" "analogs" or "chemical derivatives" of a serpin. The term "variant" is meant to refer to a molecule substantially similar in structure and function to a serpin or a part thereof. A molecule is "substantially similar" to a serpin if both molecules have substantially similar structures or if both molecules possess similar biological activity. The term "analog" refers to a molecule substantially similar in function to a serpin molecule. The term "chemical derivative" describes a molecule that contains additional chemical moieties that are not normally a part of the base molecule. A serpin may be obtained from natural or non-natural sources (e.g. recombinant or transgenic) and it may be obtained from commercial sources.

In an embodiment, the serpin is antithrombin III which may be plasma derived (see for example, U.S. Patent No. 4,087,415), transgenic (see for example, U.S. Patent No. 6,441,145), or recombinant (see for example, U.S. Patent No. 4,632,981).

The term "glycosaminoglycan" refers to linear chains of largely repeating disaccharide units containing a hexosamine and uronic acid. The precise identity of the hexosamine and uronic acid may vary widely. The disaccharide may be optionally
5 modified by alkylation, acylation, sulfonation (O- or N-sulfated), sulfonylation, phosphorylation, phosphonylation and the like. The degree of such modification can vary and may be on a hydroxyl group or an amino group. Most usually the C6 hydroxyl and the C2 amino are sulfated. The length of the chain may vary and the glycosaminoglycan may have a molecular weight of greater than 20,000 daltons,
10 typically up to 100,000 daltons, and more typically less than 50,000 daltons. Glycosaminoglycans are typically found as mucopolysaccharides. Representative examples of glycosaminoglycans include, heparin, low molecular weight heparin, dermatan sulfate, heparan sulfate, chondroitin-6-sulfate, chondroitin-4-sulfate, keratan sulfate, chondroitin, hyaluronic acid, polymers containing N-acetyl monosaccharides
15 (such as N-acetyl neuraminic acid, N-acetyl glucosamine, N-acetyl galactosamine, and N-acetyl muramic acid) and the like and gums such as gum arabic, gum Tragacanth and the like. See Heinegard, D. and Sommarin Y. (1987) *Methods in Enzymology* 144:319-373.

In an embodiment, the glycosaminoglycan is heparin. The heparin may have a
20 molecular weight in the range 6,000 to 30,000.

The invention also contemplates the use of conjugates or complexes comprising a serpin associated with a glycosaminoglycan. The term "associate", "association" or "associating" refers to a condition of proximity between a group of a glycosaminoglycan and a serpin or serpin derivative, or parts or fragments thereof.
25 The association may be non-covalent i.e. where the juxtaposition is energetically favored by for example, hydrogen-bonding, van der Waals, or electrostatic or hydrophobic interactions, or it may be covalent.

The methods of the present invention preferably use an antithrombin and heparin covalent conjugate (i.e. ATH) as described in U.S. Patent Nos. 6,491,965 and

6,562,781, Klement et al. *Biomaterials* 23:527-535, 2002 and in Berry L., Andrew M. and Chan A. K. C. *Antithrombin-Heparin Complexes* (Chapter 25). In: *Polymeric Biomaterials. Part II: Medical and Pharmaceutical Applications of Polymers*. (Second Edition) Ed. S. Dumitriu. Marcel Dekker Inc., New York, pp. 669-702, 2001, and in
5 copending US application Serial No. 60/448,116 filed February 20, 2003, which are incorporated herein in their entirety by reference. The antithrombin in ATH may be derived from plasma (see for example, U.S. Patent No. 4,087,415), it may be transgenic (see for example, U.S. Patent No. 6,441,145), or recombinant (see for example, U.S. Patent No. 4,632,981). Heparin may be obtained from pig intestine or
10 bovine lung or it may be obtained from commercial sources. Preferably, the heparin is a "high affinity" heparin enriched for species containing more than one copy of the pentasaccharide. The heparin may have a molecular weight in the range 6,000 to 30,000.

ATH is a covalent complex between antithrombin (AT) and heparin (H), and
15 therefore has a more rapid onset of action than heparin or antithrombin alone. For antithrombin to bind to, and inactivate thrombin, it must first be rendered active through the binding of heparin through a specific pentasaccharide sequence. In the ATH molecule, antithrombin is in the active conformation, ready to bind to and inactivate thrombin, thereby inhibiting clot formation.

20 ATH has improved potency over heparin because all of the heparin chains in ATH are active. In unfractionated heparin, only 33% of the heparin chains contain a pentasaccharide sequence, (the part of the heparin chain which binds to, and activates antithrombin), while only approximately 1% contain two pentasaccharide sequences. In contrast, in the ATH complex, all the heparin chains contain at least one
25 pentasaccharide sequence, and 25 to 50% of heparin chains contain two pentasaccharide sequences. In addition, unlike heparin, ATH effectively inhibits clot-bound thrombin, which is an important mediator of clot growth.

"Pentasaccharide" or "pentasaccharide sequence" refers to a key structural unit of heparin that consists of three D-glucosamine and two uronic acid residues. The

central D-glucosamine residue contains a unique 3-O-sulfate moiety. The pentasaccharide sequence represents the minimum structure of heparin that has high affinity for antithrombin (Choay, J. et al., Biochem Biophys Res Comm 1983; 116: 492-499).

5 In particular aspects, the methods, applications and compositions of the invention may utilize:

 (a) A covalent conjugate composition comprising glycosaminoglycans linked by covalent linkages to a species comprising at least one primary amino group, wherein said species is directly covalently linked via said amino group to a terminal
10 aldose residue of said glycosaminoglycans, said covalent linkages comprising an alpha-carbonyl amine formed by a substantial amount of subsequent Amadori rearrangement of imines resulting from reaction between said amino group and said terminal aldose residue of said glycosaminoglycans, or a pharmaceutically acceptable salt thereof, wherein said glycosaminoglycans are heparin (H) and said amino-
15 containing species is antithrombin III (AT).

 In an embodiment, the covalent linkage comprises a -CO-CH₂-NH- group formed by Amadori rearrangement of a -HCOH-HC=N- group resulting from reaction between the amino group and the C1 carbonyl group of the terminal aldose residue. In another embodiment of a conjugate that may be used in the present invention, the
20 molar ratio of amino-containing species to glycosaminoglycan is less than one. In a further embodiment of a conjugate that can be used in the present invention the linkages comprise an alpha-carbonyl amine formed by essentially complete subsequent Amadori rearrangement.

 (b) A covalent conjugate composition comprising glycosaminoglycans
25 and molecules comprising at least one amino group, wherein said amino group is directly linked to said glycosaminoglycans by covalent linkages, wherein said conjugate composition is made by the process comprising:

 (i) incubating said glycosaminoglycans with said molecules at a pH and for a time sufficient for imine formation between said amino group and a terminal

aldose residue of said glycosaminoglycans, and at a time and temperature sufficient for said imines to undergo a substantial amount of subsequent Amadori rearrangement to an alpha-carbonyl amine forming said covalent linkages;

(ii) isolating said conjugate composition,

- 5 or a pharmaceutically acceptable salt thereof, wherein said glycosaminoglycans are heparin (H) and said amino-containing molecules are antithrombin III (AT).

In an embodiment of a conjugate that may be used in the present invention, the molar ratio of amino-containing species to glycosaminoglycan is less than one. In another embodiment of a conjugate that may be used in the present invention the imine has undergone essentially complete subsequent Amadori rearrangement, and in
10 a particular embodiment, essentially all of the imines have undergone subsequent Amadori rearrangement. In another embodiment of a conjugate used in the invention the incubation in step (i) is carried out from about 3 days to two weeks at a temperature of 35°C to 45 °C. In another embodiment, of a conjugate used in the
15 invention the incubation in step (i) is carried out for about two weeks, more particularly 10 days.

(c) A conjugate composition comprising a substantial amount of glycosaminoglycans covalently bonded to an amino-containing species by -CO-CH₂ - NH-, said CO-CH₂- portion being derived from said glycosaminoglycan and said -NH
20 portion being derived from an amino group of said species, wherein said glycosaminoglycans are heparin (H) and said amino-containing species is antithrombin III (AT).

In an embodiment, the conjugate composition is characterized by one or more of the following: (i) the molar ratio of amino-containing species to glycosaminoglycan
25 is less than one; (ii) the conjugate has a longer half-life than heparin; (iii) it is more effective at inhibiting thrombin than are free ATIII and heparin; (iv) the conjugate inactivates clot-bound thrombin; (v) the molar ratio of heparin to antithrombin is 1:1; (vi) the molecular weight of the conjugate is 69 kD-100 kD; (vii) the conjugate possesses >60%, >90%, >95%, or >98% the antithrombin activity of intact

unconjugated heparin; and (viii) essentially all the composition comprises glycosaminoglycans.

(d) A conjugate composition comprising a substantial amount of a complex of the formula: glycosaminoglycan CO-CH₂-NH-protein, wherein the glycosaminoglycan is heparin (H) and the protein is antithrombin III (AT). In an embodiment, the molar ratio of protein to glycosaminoglycan is less than one. In another embodiment, essentially all the composition comprises glycosaminoglycan CO-CH₂-NH-protein.

“Emboli” refers to particulate matter found in subjects after medical or surgical procedures which can result in a neurological event. An emboli is generally less than 500 microns in diameter. In an aspect of the invention the emboli are generated from blood elements. In an embodiment of the invention, the emboli are thromboemboli. Emboli may be identified using conventional techniques including but not limited to transcranial or transarterial Doppler [where emboli are detected as high-intensity transient signals (HITS)], transesophageal echocardiography, and retinal fluorescein angiography.

The term “therapeutically effective dosage” as used in the present invention refers to a dosage which provides effective prevention or reduction of neurological events or provides protection or preservation of neuronal function for mammals, in particular humans, for the medical conditions and procedures described herein. In an embodiment, a therapeutically effective dosage is an amount effective to prevent or reduce emboli, in particular thromboemboli. As described herein, in general a therapeutically effective dosage in a method or composition of the invention may comprise a dosage ranging between approximately, 0.05 to 100 mg/kg, in particular 0.1 to 50 mg/kg, 0.1 to 20 mg/kg, or 1 to 10 mg/kg, more particularly 2 to 6 mg/kg. The glycosaminoglycan and serpin, including conjugates and complexes thereof may be given in 0.1 to 10 mg single intravenous boluses or 0.1 to 1.0 mg/kg intravenous boluses administered at intervals of, for example, every few seconds to several

minutes, up to a total dose of 10 – 20 mg/kg.

A “neurological event” refers to an injury to the central nervous system during or following a medical procedure including but not limited to stroke (focal neurological signs), neurophysiological impairment (subjects are obtunded, sleepy, or delirious) and encephalopathy (abnormalities in thought processes and behaviour). In an aspect of the invention, a neurological event is associated with embolization (embolism). In another aspect the neurological event is a neurocognitive deficit.

As described herein the present invention comprises a glycosaminoglycan and a serpin, including complexes and conjugates of same (in particular ATH), and methods for their use. The invention includes therapeutic applications of a glycosaminoglycan and a serpin, including complexes and conjugates of same, as neuroprotective agents. The present invention also provides a method for using a glycosaminoglycan and a serpin, including complexes and conjugates of same, in cardiac surgery which improves neurological outcome.

An aspect of the invention relates to a therapeutic application of a glycosaminoglycan and a serpin, including complexes and conjugates of same, to provide protection to a subject against neurological events, or reduce such neurological events. In an embodiment, the neurological events include but are not limited to neurological events associated with cardiac embolization.

In an aspect the invention provides a method of preventing or reducing neurological events in a subject comprising or consisting essentially of administering a therapeutically effective dosage of a glycosaminoglycan and a serpin, including complexes and conjugates of same, to the subject to prevent or reduce the neurological events.

A glycosaminoglycan and a serpin, including complexes and conjugates of same, can be administered in a therapeutically effective dosage to a subject prior to, during, or after a procedure that may give rise to a neurological event.

In another aspect, the present invention relates to a therapeutic application of a

glycosaminoglycan and a serpin, including complexes and conjugates of same, to provide protection to an individual's brain prior to scheduled, or unscheduled, procedures that may affect the cerebral circulation and/or brain. In an embodiment of the invention, a glycosaminoglycan and a serpin, including complexes and conjugates of same, are administered in a therapeutically effective dosage to a subject prior to, during, or after a procedure that may affect the central nervous system, in particular the brain and/or cerebral circulation.

In another aspect of the invention, a glycosaminoglycan and a serpin, including complexes and conjugates of same, are administered in a therapeutically effective dosage into the circulation or into the brain ventriculocisternal (fluid circulation) system of a subject prior to, during, or after a procedure that may affect the central nervous system, in particular the brain and/or cerebral circulation.

As described herein a glycosaminoglycan and a serpin, including complexes and conjugates of same, are administered in a therapeutically effective dosage into the circulation or into the brain ventriculocisternal (fluid circulation) system prior to a procedure that may affect the central nervous system, in particular the brain and/or cerebral circulation.

A method of protecting neuronal function in vivo in the central nervous system, in particular the brain and/or the cerebral circulation, is provided comprising the step of administering to a subject a therapeutically effective dosage of a glycosaminoglycan and a serpin, including complexes and conjugates of same, prior to a medical or surgical procedure.

In general, the methods, therapeutic applications, and compositions of the invention may be used with any medical or surgical procedure that may give rise to a neurological event including procedures for coronary artery diseases, valvular heart disease, congenital heart disease, aortic disease, transplantation and a variety of other procedures. Examples of such procedures include but are not limited to surgical procedures, for example cardiopulmonary bypass, cardiac catheterization, angioplasty, endarterectomy, and other medical procedures that may affect cerebral circulation. A

procedure may also include the administration of pharmaceutical compositions that may affect cerebral circulation. It will be appreciated that the therapeutic applications for the glycosaminoglycan and serpin described herein are by no means limited to the disclosed medical conditions but instead include other conditions that will be apparent to those skilled in the art.

In particular aspects, the methods of the invention can be used to prevent cerebral embolization and to prevent or reduce emboli in the cerebral circulation and/or brain. The methods can be employed on various patients, in particular, those at high risk for cerebral embolization, in order to reduce the risk for cerebral embolization which can lead to neurologic or cognitive complications and death.

In an embodiment, the invention provides a method for reducing emboli (in particular, thromboemboli) in the cerebral circulation in a subject comprising administering an amount of a glycosaminoglycan and a serpin, including complexes and conjugates of same, effective to reduce the emboli.

The invention also relates to a method of cerebral embolic protection in a subject comprising administering an amount of a glycosaminoglycan and a serpin, including complexes and conjugates of same, to reduce emboli in the cerebral circulation.

Further, the invention relates to a method for protecting a subject against cerebral embolization comprising administering an amount of a glycosaminoglycan and a serpin, including complexes and conjugates of same, that reduces the amount of emboli that reach the cerebral vasculature.

The invention also provides methods for eliminating or minimizing cerebral embolization during invasive cardiac procedures.

In particular, a glycosaminoglycan and a serpin, including complexes and conjugates of same, are administered to a subject prior to, during, or after a cardiopulmonary bypass procedure. A complication of cardiopulmonary bypass is the formation of emboli that lodge within the cerebral blood vessels resulting in local

areas of blood flow cessation or ischemia. In accordance with a method of the invention, administration of a glycosaminoglycan and a serpin, including complexes and conjugates of same, prior to, during or after the bypass procedure can reduce the likelihood of neurological problems. A glycosaminoglycan and a serpin, including
5 complexes and conjugates of same, may be used with other planned surgical procedures where emboli are released into the brain circulation or transient disruption of blood flow to the brain occurs, including but not limited to carotid endarterectomy, clipping of aneurysms, etc.

The invention provides a method of preventing or reducing emboli from a
10 bypassed heart region prior to removal of the region from bypass comprising administering an amount of a glycosaminoglycan and a serpin, including complexes and conjugates of same, effective to prevent or reduce the emboli.

The invention also provides a method for improving the outcome of cardiac surgery in a subject undergoing cardiopulmonary bypass surgery comprising
15 administering a therapeutically effective amount of a glycosaminoglycan and a serpin, including complexes and conjugates of same, effective to prevent or reduce the emboli.

A glycosaminoglycan and a serpin, including complexes and conjugates of same may be administered to a subject during induction of anesthesia, during surgery,
20 and/or after surgery. In embodiments of the invention, administration of a glycosaminoglycan and a serpin is performed after intubation of the patient.

In an aspect of the invention, the glycosaminoglycan and serpin, including conjugates and complexes thereof are administered peri-operatively. In an aspect, the agents are administered pre-sternotomy or post-sternotomy. They may be administered
25 in a continuous intravenous infusion, or a plurality of intravenous boluses. They may be administered after intubation but before placing the subject on cardiopulmonary bypass.

The invention also provides a method of performing cardiac surgery, in particular, CABG surgery, in which a therapeutically effective amount of

glycosaminoglycan and a serpin, including complexes and conjugates of same, are administered peri-operatively to a subject undergoing cardiopulmonary bypass to reduce the effects of emboli. The glycosaminoglycan and serpin, including complexes and conjugates of same, may be administered during the surgery, particularly after
5 intubation for general anesthesia. They may be administered as a continuous infusion or multiple boluses.

In certain aspects of the invention, the glycosaminoglycan and serpin provide synergistic activity in preventing or reducing neurological events. Thus, a method of preventing or reducing cerebral emboli in a patient is provided comprising or
10 consisting essentially of administering to a patient in need thereof, synergistically effective amounts of a glycosaminoglycan and a serpin. By "synergistic activity" or "synergistically effective amount" is meant that a sufficient amount of glycosaminoglycan and serpin will be present in order to achieve a desired result that is greater than the result achieved with each component on its own, e.g. improved
15 reduction of neurological events.

In certain aspects of the invention a glycosaminoglycan and a serpin are administered in combination. In particular, they can be administered concurrently to a patient being treated. When administered in combination, each component may be administered at the same time or sequentially in any order, and at different points in
20 time. Therefore, each component may be administered separately, but sufficiently close in time to provide the desired effect (in particular, a synergistic effect). The components may be associated, for example, they may form a complex or conjugate. In a particular embodiment, the components form ATH.

The present invention also provides compositions comprising or consisting
25 essentially of a combination of therapeutically effective amounts of glycosaminoglycan and a serpin, including conjugates and complexes thereof, together with a pharmaceutically acceptable excipient, carrier, or vehicle.

Also contemplated is a pharmaceutical composition in separate containers and intended for simultaneous or sequential administration, comprising a

glycosaminoglycan and a serpin, both together with pharmaceutically acceptable excipients, carriers, or vehicles.

In another embodiment, the invention provides a pharmaceutical composition comprising a unit dosage of a glycosaminoglycan, and a unit dosage of a serpin,
5 together with a pharmaceutically acceptable excipient, carrier, or vehicle.

The above mentioned compositions and treatments also include pharmaceutically acceptable salts of the glycosaminoglycan and the serpin, such as sodium, potassium, ammonia, magnesium, and calcium salts.

In accordance with one aspect, a pharmaceutical composition is provided
10 comprising a glycosaminoglycan and a serpin effective to exert a synergistic effect in preventing or reducing neurological events in particular neurological events associated with emboli, more particularly cerebral embolization. The invention also provides pharmaceutical compositions comprising a synergistically effective amount of a combination of a glycosaminoglycan and a serpin in a pharmaceutically acceptable
15 excipient, carrier, or vehicle.

In another aspect the invention relates to a method of using a composition comprising a glycosaminoglycan and a serpin, including complexes and conjugates of same, in the preparation of a medicament for preventing or reducing neurological events in particular neurological events associated with emboli, more particularly
20 cerebral embolization.

In a further aspect the invention relates to a method of using synergistically effective amounts of a glycosaminoglycan and a serpin in the preparation of a pharmaceutical composition for preventing or reducing neurological events, in particular neurological events associated with emboli, more particularly cerebral
25 embolization.

Since some aspects of the present invention relate to a method of treatment comprising active agents which may be administered separately, the invention also relates to combining separate compositions comprising the active agents in kit form.

The invention also contemplates the use of a composition of the invention or treatment of the invention for preventing, and/or ameliorating disease severity, disease symptoms associated with neurological events, in particular neurological events associated with emboli.

5 Subjects or patients that may receive a treatment or be administered a composition of the invention include animals, including mammals, and particularly humans. Animals also include domestic animals, including horses, cows, sheep, poultry, fish, pigs, cats, dogs, and zoo animals.

10 A serpin and a glycosaminoglycan, including conjugates or complexes thereof (e.g. in particular ATH) can be administered by any means that produce contact of an active agent with the agent's sites of action in the body of the patient. The substances can be administered simultaneously or sequentially in any order, and at different points in time, to provide the desired effect. It lies within the capability of a skilled physician or veterinarian to chose a dosing regime that optimizes the effects of the
15 compositions and treatments of the present invention. The compositions may be administered in such oral dosage forms as tablets, capsules (each of which includes sustained release or timed release formulations), pills, powders, granules, elixirs, tinctures, suspensions, syrups, and emulsions. They may also be administered in intravenous (bolus or infusion), intraperitoneal, subcutaneous, or intramuscular form,
20 all using dosage forms well known to those of ordinary skill in the pharmaceutical arts. The compositions of the invention may be administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes, for example using conventional transdermal skin patches. The dosage administration in a transdermal delivery system will be continuous rather than intermittent throughout the dosage
25 regimen.

The dosage regimen will vary depending upon known factors such as the pharmacodynamic characteristics of the particular agent and its mode and route of administration; the species, age, sex, health, medical condition, and weight of the patient, the nature and extent of the symptoms, the kind of concurrent treatment, the

frequency of treatment, the route of administration, the renal and hepatic function of the patient, and the desired effect. The effective amount of a drug required to prevent, counter, or arrest progression of a condition can be readily determined by an ordinarily skilled physician or veterinarian.

5 A composition or treatment of the invention may comprise a unit dosage of a glycosaminoglycan and a serpin. A "unit dosage" refers to a unitary i.e. a single dose which is capable of being administered to a patient, and which may be readily handled and packed, remaining as a physically and chemically stable unit dose comprising either the active agent as such or a mixture of it with solid or liquid pharmaceutical
10 excipients, carriers, or vehicles.

 The glycosaminoglycan, serpin, complexes and conjugates thereof, compositions of the present invention or components thereof typically comprise suitable pharmaceutical diluents, excipients, vehicles, or carriers selected based on the intended form of administration, and consistent with conventional pharmaceutical
15 practices. The carriers, vehicles etc. may be adapted to provide a synergistically effective amount of the active components to prevent or reduce neurological events.

 Suitable pharmaceutical diluents, excipients, vehicles, and carriers are described in the standard text, Remington's Pharmaceutical Sciences, Mack Publishing Company. By way of example for oral administration in the form of a
20 capsule or tablet, the active components can be combined with an oral, non-toxic pharmaceutically acceptable inert carrier such as lactose, starch, sucrose, methyl cellulose, magnesium stearate, glucose, calcium sulfate, dicalcium phosphate, mannitol, sorbital, and the like. For oral administration in a liquid form, the drug components may be combined with any oral, non-toxic, pharmaceutically acceptable
25 inert carrier such as ethanol, glycerol, water, and the like. Suitable binders (e.g. gelatin, starch, corn sweeteners, natural sugars including glucose; natural and synthetic gums, and waxes), lubricants (e.g. sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, and sodium chloride), disintegrating agents (e.g. starch, methyl cellulose, agar, bentonite, and xanthan gum), flavoring agents, and

coloring agents may also be combined in the compositions or components thereof.

Formulations for parenteral administration of a composition of the invention may include aqueous solutions, syrups, aqueous or oil suspensions and emulsions with edible oil such as cottonseed oil, coconut oil or peanut oil. Dispersing or suspending agents that can be used for aqueous suspensions include synthetic or natural gums, such as tragacanth, alginate, acacia, dextran, sodium carboxymethylcellulose, gelatin, methylcellulose, and polyvinylpyrrolidone.

Compositions for parenteral administration may include sterile aqueous or non-aqueous solvents, such as water, isotonic saline, isotonic glucose solution, buffer solution, or other solvents conveniently used for parenteral administration of therapeutically active agents. A composition intended for parenteral administration may also include conventional additives such as stabilizers, buffers, or preservatives, e.g. antioxidants such as methylhydroxybenzoate or similar additives.

A composition or component thereof may be sterilized by, for example, filtration through a bacteria retaining filter, addition of sterilizing agents to the composition, irradiation of the composition, or heating the composition. Alternatively, the active ingredients may be provided as sterile solid preparations e.g. lyophilized powder, which is readily dissolved in sterile solvent immediately prior to use.

In addition to the formulations described previously, the compositions and components thereof can also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example, subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the agents may be formulated with suitable polymeric or hydrophobic materials (for example, as an emulsion in an acceptable oil), or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. For administration of a composition of the invention, such labeling would include amount, frequency, and method of administration.

The present invention also includes methods of using the compositions of the invention in combination with one or more additional therapeutic agents including without limitation anti-platelet or platelet inhibitory agents such as aspirin, piroxicam, clopidogrel, ticlopidine, or glycoprotein IIb/IIIa receptor antagonists, thrombin inhibitors such as heparin, boro-peptides, hirudin, or argatroban; or thrombolytic or fibrinolytic agents, such as plasminogen activators (such as tissue plasminogen activator), anistreplase, urokinase, or streptokinase; or combinations thereof.

The invention will be described in greater detail by way of a specific example. The following example is offered for illustrative purposes, and is not intended to limit the invention in any manner. Those of skill in the art will readily recognize a variety of noncritical parameters which can be changed or modified to yield essentially the same results.

Example 1

Summary

The purpose of this study was to examine the efficacy of an antithrombin-heparin covalent complex (ATH) and equivalent doses of either unfractionated heparin or unfractionated heparin supplemented with transgenic antithrombin in a pig cardiopulmonary bypass (CPB) model.

The test substances were injected at several doses as an iv bolus after sternotomy. About 20 minutes after injection, CPB was begun with hypothermic lowering of the bypass blood temperature to 28°C. Bypass was continued for 2 hours, and normothermia re-established in the last 45 minutes. After bypass, neutralizing protamine sulfate was administered, followed by a 3 hour recovery. Throughout the experiment, microemboli were monitored using arterial ultrasound Doppler HITS (High Intensity Transient Signals) as a primary end point. ACT, chest blood accumulation, protein deposition in the bypass circuit, TATs, and D-dimers were monitored as secondary end points.

ATH reduced the HIT rate during CPB, and the reduction was dose dependant.

The majority of HITS appeared to represent microemboli, and ATH reduced microemboli formation, particularly at a dose of 3mg/kg. In all cases, complete anticoagulation reversal was achieved by standard dosing of protamine sulfate.

5 **Methods and Experimental Design**

TEST SYSTEM

Pig thrombosis models have been used to evaluate anticoagulants for many years. The effects of anticoagulants in pig models have proven to be predictive of their efficacy in human thrombotic conditions (Munster, Am et al, Comp. Med. 52: 39-43, 10 2002).

Description of Test System

30, 32-66 kg female Yorkshire pigs were obtained from the University of Guelph Arkell Research Center (Canada) and acclimatized for at least one week prior to the study.

15 **Test Animal Housing**

Pigs were housed at the Hamilton Research Center Animal Facility, and animals had ad libitum access to autoclaved Purina Porcine Lab Diet (#5084). The animal room environment and photoperiod was controlled to target conditions of 20°C, 50% humidity & 12 hr light/12 hr dark.

20 **Test Anticoagulants**

Heparin of various lots (injection sodium heparin from Organon Teknika Inc.), ATH of various lots (HRC), and human recombinant antithrombin (AT) of transgenic source (Genzyme Transgenics) were used.

The Animal Model (Figure 1)

25 Anesthesia was initiated by intramuscular injection of ketamine (20mg/kg), acepromazine (0.2mg/kg), and atropine (0.05mg/kg) into Yorkshire pigs and maintained with a mixture of isoflurane (1-3%), oxygen (1.8L/min), and nitrous

oxide (1.2L/min) delivered through a 9.5F endotracheal tube using a positive pressure respirator. The respiratory rate was adjusted to maintain arterial blood pH, pCO₂, and pO₂ in the physiologic range. During CPB, inhalation anesthesia is delivered through the membrane oxygenator and supplemented with 1-2ml of iv sodium Phenobarbital.

- 5 The total anaesthesia time was about 6 hours (1 hour of pre-CPB surgical manipulation, 2 hours of CPB, 3 hours of post-CPB recovery).

A 14 gauge iv cannula was inserted in the marginal auricular vein of the right ear for administration of drugs and fluids, and the femoral artery and both carotid arteries were exposed. A blood pressure transducer was connected to the right femoral artery, while Doppler ultrasound transducer probes were placed on both carotid arteries. "Pre" CPB HIT data were collected, and a "baseline" ACT was measured. A 5mg/kg bolus of bretylium tosylate was administered intravenously to prevent cardiac arrhythmia, and the heart and great vessels were exposed through a median sternotomy. A pericardial cradle was then created and hemostasis was secured.

- 15 The test anticoagulant drug was administered (approximately 50 minutes after taking the "baseline" ACT and more than 20 minutes after sternotomy). 5 minutes later, a blood sample was taken for ACT. An ACT of at least 500 seconds was required before proceeding. If this ACT value was not achieved at the beginning or at any time during CPB, the anticoagulant was supplemented with ¼ doses until the ACT exceeded 500 seconds (supplementation of AT+heparin was with heparin only). The ascending aorta was cannulated and connected to the CPB circuit (for "partial bypass"), taking care to avoid air bubbles, and then the right auricle was cannulated to secure a venous line to the CPB circuit. CPB began approximately 20 minutes after injection of the anticoagulant. Reduction of core body temperature to 28°C was initiated and maintained using the CPB circuit heater/cooler unit (the hypothermic state was reached about 20 minutes into CPB).

The CPB circuit was composed of the following components: an affinity integrated CVR membrane oxygenator with a heater/cooler unit, a venous reservoir (maintained at 800ml with either Ringer's lactate solution containing 7.5% (44mEq)

sodium bicarbonate or reused cavity blood), an on-line arterial blood filter, a roller pump set for 3.5-4L/min (60% of cardiac output), a 2 stage armored venous drainage catheter, and a return arterial cannula.(See Figure 2) Mean arterial pressure was maintained above 60mm Hg. A suction pump was also available, but it was turned off
 5 during the bypass to allow manual measurement of chest cavity bleeding. After periodic measurement of cavity blood, the blood was returned to the CPB system via the oxygenator reservoir.

Periodic blood sampling was performed for blood gases, pH analysis, ACT (t_0 set at the beginning of bypass), anticoagulant levels, CBC, hematocit, and TAT levels.
 10 Supportive therapy was instituted if needed (epinephrine, dopamine, CaCl_2 , Na bicarbonate, etc.), and supplementation of the anticoagulant with $\frac{1}{4}$ doses was given if the ACT decreased below 500 seconds. Chest blood volume was periodically measured as well as rectal temperature.

After about $1\frac{1}{4}$ hours of hypothermic CPB, warm up of the pig was begun, and
 15 after a total CPB time of 2 hours, the venous CPB line was removed, and remaining blood from the CPB circuit was infused. Decannulation took about 5 minutes, after which a "Pre-Protamine" ACT was taken.

After 5 minutes, anticoagulation was reversed with protamine sulphate to reach the pre-CPB ACT value (50mg of protamine sulphate, typically used to neutralize
 20 5,000 IU of heparin, was used in this model for 50Kg pigs given an initial dose of 300U heparin/Kg, based on previous experience). When stabilization was achieved, the CPB arterial line was removed. Two chest tubes were placed into the pericardial cavity, connected to the chest drainage unit, and kept under negative pressure of 10 ml water. An ACT was then taken (protamine t_0).

25 After 3 hours post-CPB (post-protamine), animals were anticoagulated with heparin and then euthanized with sodium phenobarbital. For every pig, the brain, tissue samples from primary soft tissue organs, and a skin sample were saved and stored in 10% buffered formalin.

Selection of Doses

The doses of anticoagulants, were chosen to cover the maximum range that might be encountered in CPB.

Animal Identification and Treatment Groups

A total of 30 pigs, individually identified by numbered ear tags following
 5 arrival, were assigned to 6 Groups. Pigs were dosed with the concentrations of
 anticoagulants as specified in Table 1. Doses shown on the graphs in the Figures are
 the initial doses (not final doses when supplementation was required).

Observations

10 Doppler HITS

Trans-arterial ultrasound Doppler HITS were measured by placing two round
 2MHz probes (Spencer Technologies) liberally covered with Aquasonic 100
 ultrasound transmission gel (Parker Labs) on each of the two carotid arteries. Care
 was taken to avoid air bubbles in the gel, and the probes were oriented at a 20-degree
 15 angle with respect to the artery. Adjustments were made to optimize the signal, which
 was sent to a computer in the TCD 2020 transcranial Doppler machine (Nicolet
 Vascular) for digitized storage and computer recognition of HITS.

Discrimination of microemboli from micro air bubbles and dislodged fat was
 investigated in a preliminary study, and distinctive patterns characteristic of each
 20 agent were seen. In this study, this level of discrimination was not needed, so all
 HITS were counted.

HITS were integrated for segments of time represented by the blood sampling
 times shown on the time-line (Figure 1). These integrated values were then analyzed
 by three methods. Total HITS were summed for the study segments "Pre-CPB",
 25 "CPB", and "Post-CPB", an average hit rate (normalized per hour) was determined for
 each of these study segments, and normalized hit rates were determined for smaller
 sampling segments (to give a more dynamic picture of HIT response).

Blood Loss Measurements

Chest cavity blood was collected periodically, measured for volume, and returned to the CPB circulation.

Protein Deposition Measurements

5 At the end of bypass, the filter/oxygenator/reservoir linked units were flushed with Ringer's solution, followed by 2 liters of 2M sodium hydroxide perfused in the CPB circuit for 1 hour. The sodium hydroxide wash was sampled and analyzed for total protein and hemoglobin. For hemoglobin, samples were diluted 1:10, measured at 540nm on a spectrophotometer, and the values compared to a standard curve. For
10 protein, samples were diluted 1:100 and measured with a standard commercial protein assay.

Blood Samples for Coagulation Analysis and Reference

Periodic blood samples were taken from the pig as indicated on the time-line (Figure 1) and as needed 2ml EDTA samples were taken for CBC, 5ml citrate plasma
15 samples for anticoagulant assays/TATs & D-dimer, 3ml samples for ACT, 1ml samples in pre-heparinized syringes for blood gas/pH/sodium/potassium.

Tissue Analysis

The brain, tissue samples from primary organs and a skin sample were saved and stored in formalin for every pig for potential future processing.

20 Statistical Analysis

Data means and standard errors of the mean (SEM) (derived from population standard deviations) were calculated for graphical representation.

RESULTS

Doppler HITS/HR

25 Pre-CPB HITS occurred at a rate of about 250/hr regardless of the anticoagulant given or its dose (Figure 3A). Since anticoagulants were injected well after sternotomy, it is likely that pre-CPB HITS reflect an activation of the coagulation

cascade as a result of tissue damage induced by sternotomy.

During CPB, HITS increased for UFH by almost 50% (Figure 3B), regardless of dose, and would undoubtedly be much higher if UFH were not present. A dose-dependent decrease in CPB HITS is seen with ATH at doses ranging from 1-3mg/kg. AT (3mg/kg) + H (300U/kg) resulted in an intermediate HITS rate between UFH and the 2mg/kg ATH.

When the CPB HITS rate is expressed as a percentage of the pre-CPB HITS rate (Figure 3C), the dose and agent patency relations become much clearer. Calculation of mean and SEM are done after calculation of percentage for individual pigs. This results in a more accurate assessment than simply dividing mean results from Figure 3B by those of Figure 3A. As can be seen, an expected dose-response is generated for UFH, a clearer dose-response is seen with ATH, and the intermediate value of AT+H also is clearer. In light of the fact that varying amounts of drug supplementation were required during CPB to maintain ACT values above 500 seconds (Table 1), it is interesting that the dose-response is so clear.

Average post-CPB HITS data (Figure 3D) mirror the average CPB HITS data, suggesting that the effect of test anticoagulants persists even after complete protamine reversal.

Bleeding

Bleeding (Figure 4 expressed as ml/hr) was low for all animals in this study. Therefore, all tested anticoagulants show comparable safety profiles during CPB.

Protein Deposition

Protein Deposition on the CPB circuit, measured either as total protein or as hemoglobin, is shown in Figure 5. Protein deposition is a pooled measurement from the sodium hydroxide wash of the blood reservoir (exposed to static and low flow rate blood), oxygenator, and blood filter (exposed to high flow rate blood). These data suggest that 1000U/kg UFH and 3mg/kg ATH result in little protein and fibrin accretion on the circuit, whereas 300U/kg UFH, 2mg/kg ATH, and AT+H are less

effective at reducing deposition.

ACT

ACT Group averaged dynamic representation of ACT data (Figure 7 in seconds) shows the anticoagulant effect of the various test agents and the ability of protamine sulphate to reverse these effects. The highest mean ACT levels during CPB (also requiring the least amounts of supplementation) were achieved with 1000U/kg UFH and 3mg/kg ATH. The AT+H profile was midway between the profiles for 300U/kg heparin and 1mg/kg ATH. Protamine sulphate neutralized the anticoagulant effect of all test articles, bringing the ACT back to baseline levels. Protamine reversal for all agents is most clearly seen in the graph of Figure 8.

TATs

Thrombin antithrombin complexes (TAT) (Figure 9) increase in a characteristic way during CPB and decline thereafter. It is not clear from these data whether the decline is dependent on either the termination of CPB, the neutralization of anticoagulant by protamine sulfate (not likely), or simply the time after initiation of CPB. The very low level of TATs and flat profiles during CPB (as well as the gradual increase following CPB) for 1000U/kg UFH and 3mg/kg ATH are in striking contrast to the rapid rise in TATs for AT+H and lower doses of ATH and UFH. It is also not clear from these data whether the gradual increase in TATs following CPB for 1000U/kg UFH and 3mg/kg ATH is due to protamine reversal or just coincidental.

D-dimers

D-dimer (Figure 10) levels are a less sensitive index of thrombin activation than TATs. There is no evidence of an increase in D-dimer level during CPB for 1000U/kg UFH or 3mg/kg ATH, though there is a trend toward an increase for all other agents. Protamine reversal causes a pronounced increase in the 3mg/kg ATH D-dimer level but does not affect the level for 1000U/kg UFH.

Conclusions

1. The dose-dependent CPB HITS rate decreases in response to injection of

anticoagulants. Thus, the majority of HITS represent microemboli.

2. ATH reduced the HITS rate during CPB. During CPB, UFH yields a HITS rate almost twice that seen pre-CPB, even for heparin doses as high as 1000U/kg. ATH at a dose of 3mg/kg) reduces CPB HITS rate to about half the pre-CPB rate.
5 AT+H (AT dose 3mg/kg) maintains the CPB HITS rate at an intermediate level that is higher than the pre-CPB HITS rate. Protein accretion in the bypass circuit tends to confirm the efficacy of 3mg/kg ATH and shows an equivalent lowering of protein accretion with 1000U/kg heparin.
- 10 3. All anticoagulant agents tested can be completely reversed with standard doses of protamine sulfate.

Table 1: Treatment Groups

<u>Group</u>	Anticoagulant	Animals per group	Initial heparin mass equivalent (mg/Kg)	Initial heparin activity equivalent (U/Kg)	Total heparin mass equivalent (mg/Kg)	Total heparin activity equivalent (U/Kg)	Neutralizing Prot. Sulfate given (mg/Kg)
1	300 U/Kg heparin	6	1.9	300	2.6	421	0.52
2	1000 U/Kg heparin	6	6.3	1000	6.3	100	1.94
3	1mg/Kg ATH	3	0.4	193	0.6	337	1.18
4	2mg/Kg ATH	6	0.6	384	0.8	506	1.06
5	3mg/Kg ATH	5	1.1	625	1.1	655	1.59
6	3mg/Kg AT + 300 U/Kg heparin	5	1.9	300	2.2	345	1.06

FULL CITATIONS FOR REFERENCES REFERRED TO IN THE SPECIFICATION

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The present invention is not to be limited in scope by the specific embodiments described herein, since such embodiments are intended as but single illustrations of one aspect of the invention and any functionally equivalent embodiments are within the scope of this invention. Indeed, various modifications of the invention in addition
5 to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

All publications, patents and patent applications referred to herein are incorporated by reference in their entirety to the same extent as if each individual
10 publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety. The citation of any reference herein is not an admission that such reference is available as prior art to the instant invention.

It must be noted that as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural reference unless the context clearly dictates
15 otherwise.

WE CLAIM:

1. A method of preventing or reducing neurological events in a subject comprising administering a therapeutically effective dosage of a glycosaminoglycan and a serpin to the subject to prevent or reduce the neurological events.
2. A method of claim 1 wherein the neurological event is associated with emboli.
3. A method of claim 2 wherein the emboli are thromboemboli.
4. A method of claim 1 wherein the serpin is antithrombin III.
5. A method of claim 4 wherein the glycosylaminoglycan is heparin.
6. A method of claim 1 wherein the glycosylaminoglycan and serpin form a complex or conjugate.
7. A method of claim 6 wherein the complex or conjugate comprises antithrombin III covalently linked to heparin.
8. A method of claim 7 wherein the complex or conjugate comprises ATH.
9. A method of claim 1 wherein the glycosaminoglycan and the serpin are administered prior to, during, or after a procedure that may give rise to a neurological event.
10. A method of claim 9 wherein the procedure affects the central nervous system.
11. A method of claim 10 wherein the procedure affects the brain and/or cerebral circulation.
12. A method of claim 9 wherein the procedure is a surgical procedure.
13. A method of claim 12 wherein the procedure is cardiopulmonary bypass, cardiac catheterization, angioplasty, or endarterectomy.
14. A method as claimed in claim 2 comprising reducing emboli in the cerebral circulation in a subject comprising administering an amount of a

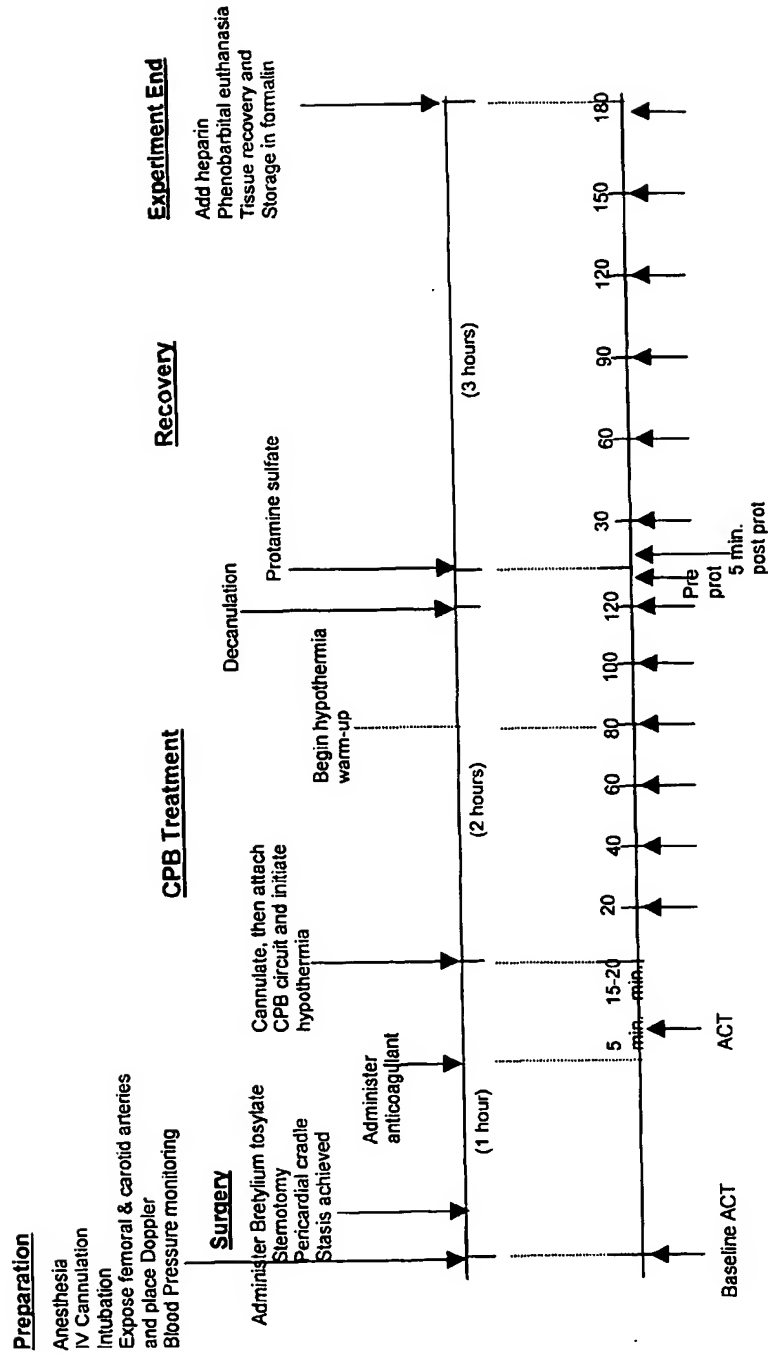
glycosaminoglycan and a serpin effective to reduce the emboli.

15. A method of claim 1 for protecting a subject against cerebral embolization comprising administering an amount of a glycosaminoglycan and a serpin that reduces the amount of emboli that reach the cerebral vasculature.
- 5 16. A method of performing cardiac surgery comprising administering a therapeutically effective amount of a glycosaminoglycan and a serpin peri-operatively to a subject undergoing cardiopulmonary bypass to prevent or reduce the effects of emboli.
- 10 17. A method of claim 16 wherein the glycosylaminoglycan and serpin form a complex or conjugate.
18. A method of claim 17 wherein the complex or conjugate comprises antithrombin III covalently linked to heparin.
19. A method of claim 18 wherein the complex or conjugate comprises ATH.
- 15 20. A method of claim 2 comprising preventing or reducing emboli from a bypassed heart region prior to removal of the region from bypass by administering an amount of a glycosaminoglycan and a serpin effective to prevent or reduce the emboli.
21. A method of using a glycosaminoglycan and a serpin in the preparation of a medicament for the prevention or inhibition of cerebral embolization.
- 20 22. A method of using synergistically effective amounts of a glycosaminoglycan and a serpin in the preparation of a pharmaceutical composition for preventing or reducing neurological events.
23. A pharmaceutical composition comprising a combination of a glycosaminoglycan and a serpin effective to exert a synergistic effect in
25 preventing or reducing neurological events.

ABSTRACT OF THE DISCLOSURE

The present invention relates to therapeutic methods for preventing or reducing neurological events utilizing a glycosaminoglycan and a serpin, including complexes and conjugates thereof. Methods of the present invention may be advantageous for preventing or reducing neurological events prior to, or during medical or surgical procedures, and after a neurological event. In particular, the present invention deals with neurological events associated with the generation of emboli that can lodge in the brain and/or cerebral circulation during cardiac surgery.

Pig CPB Model



Periodic blood samples and as needed (2ml EDTA samples for CBC, 5ml citrate plasma samples for anticoagulant assays/TATs & D-dimer, 3ml samples for ACT, 1ml samples in heparinized syringe for blood gas)

FIGURE 1

Figure 2

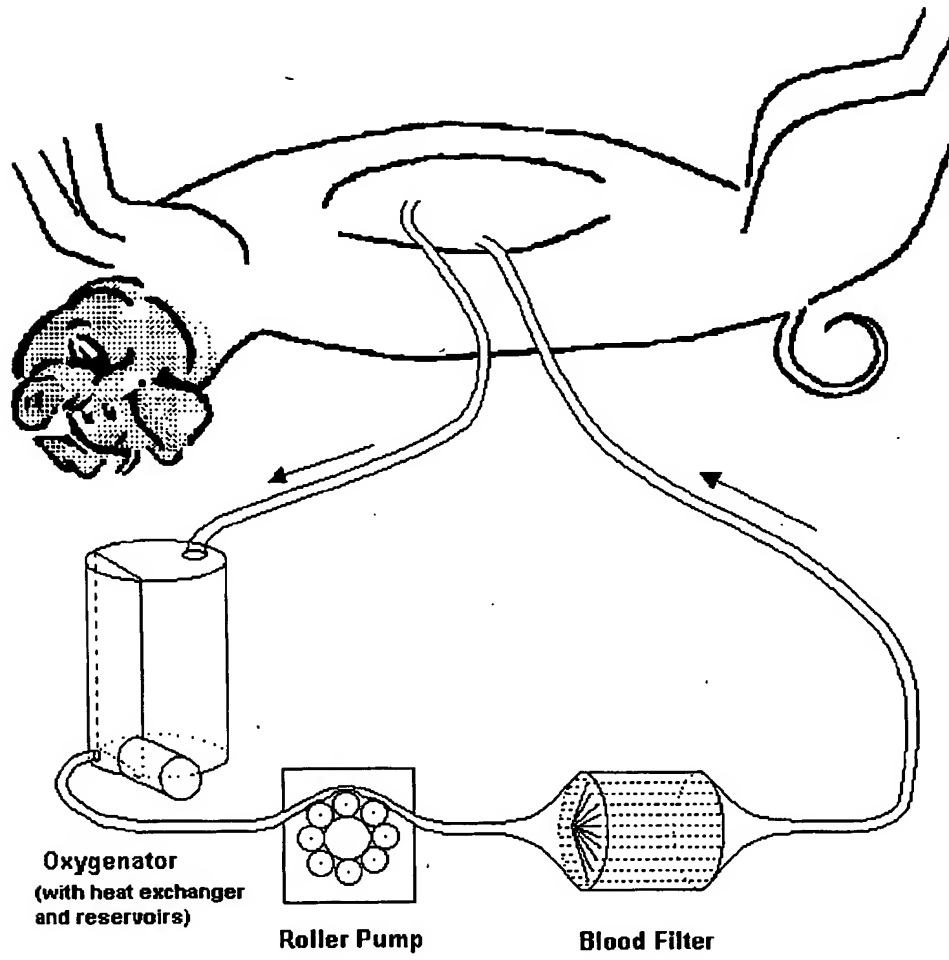


Figure 3

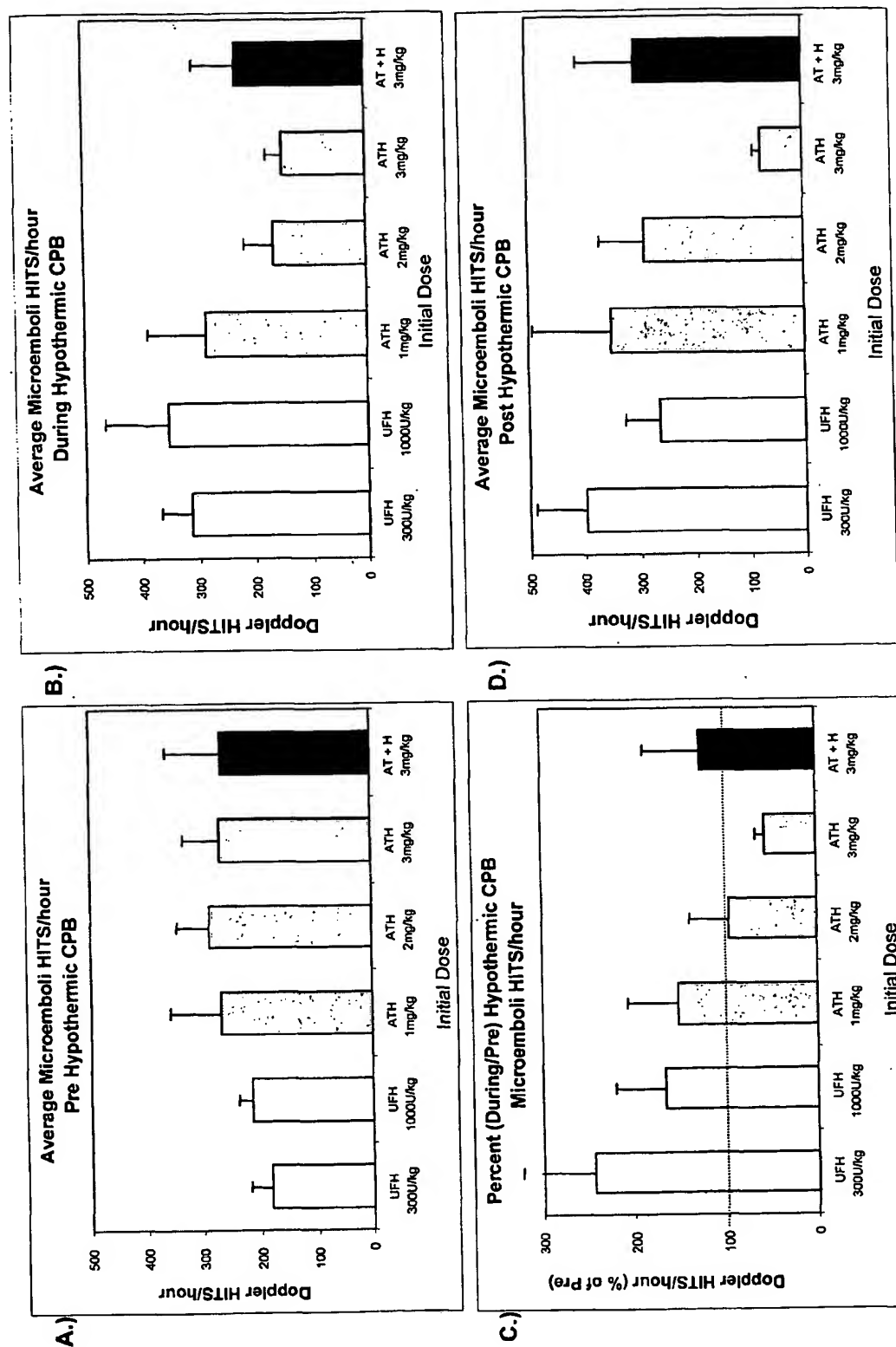


Figure 4

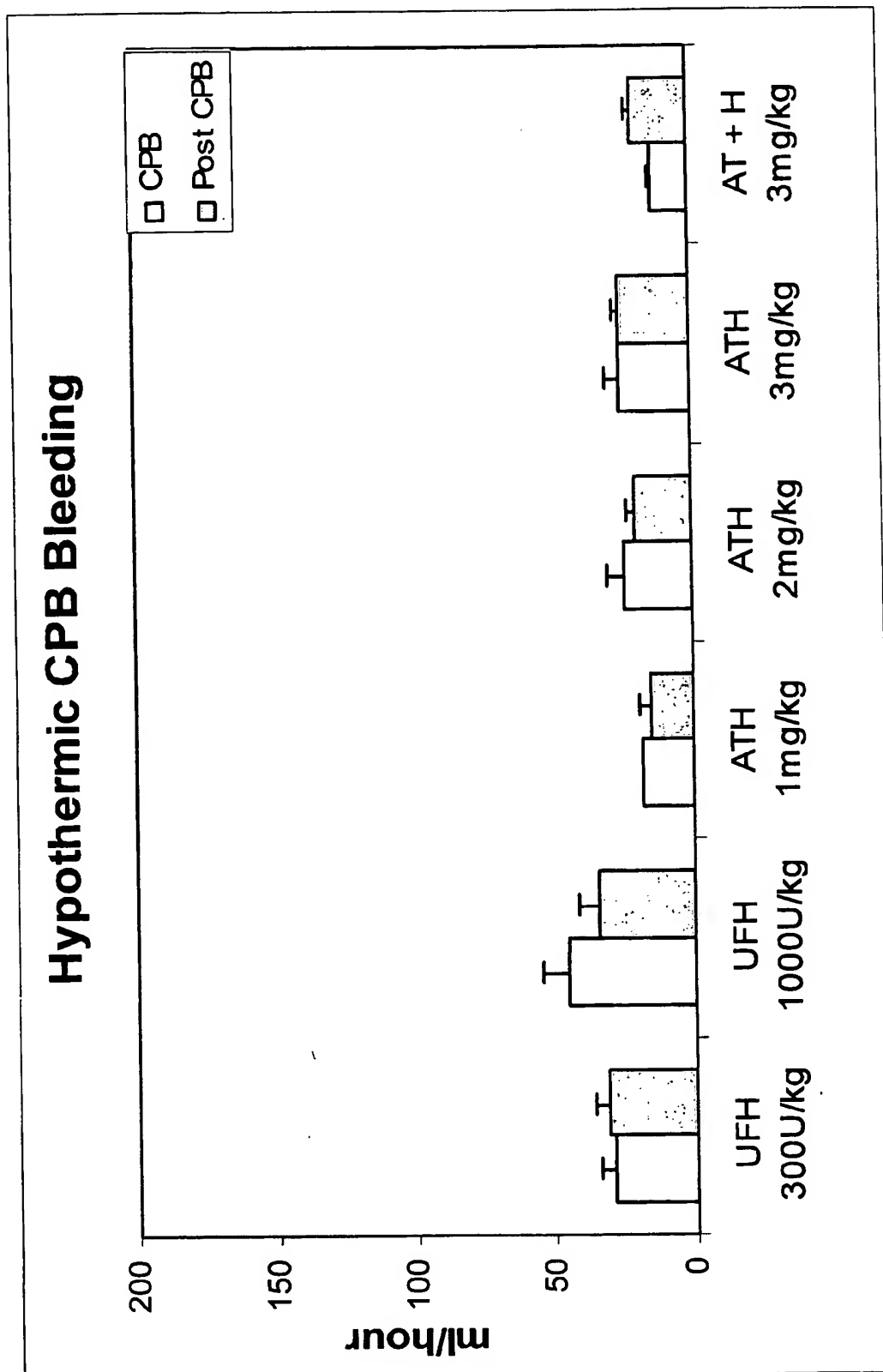


Figure 5

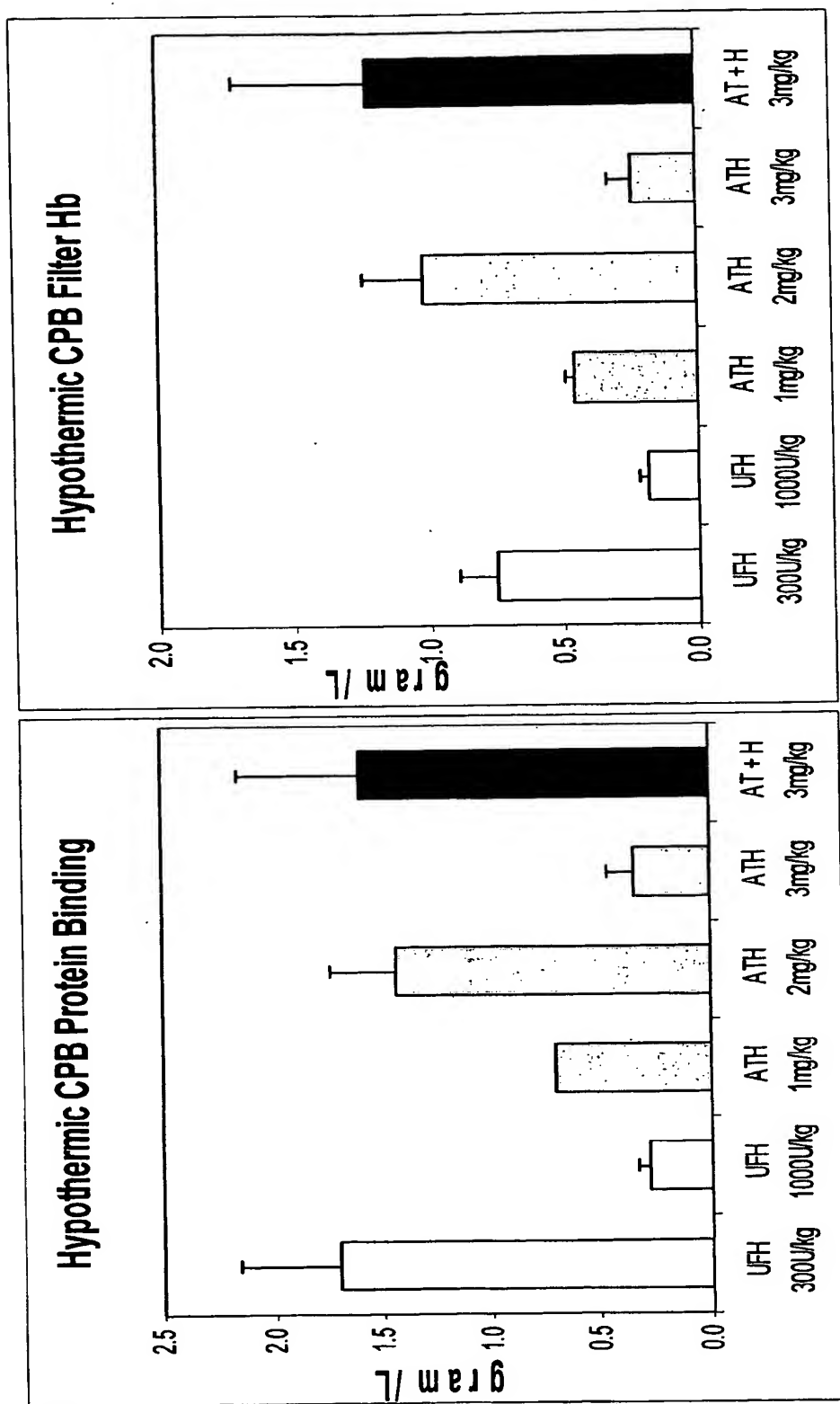
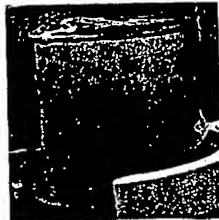


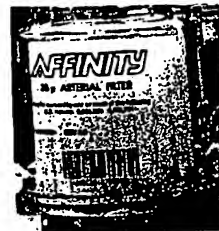
Figure 6

Arterial side
Blood Filter

Venous side
Cardiotomy Reservoir



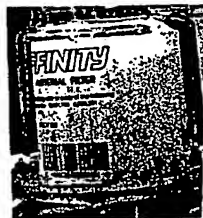
H 300



H 1000



AT + H



ATH (2)



ATH (3)

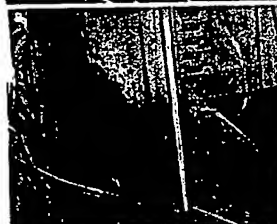


Figure 7

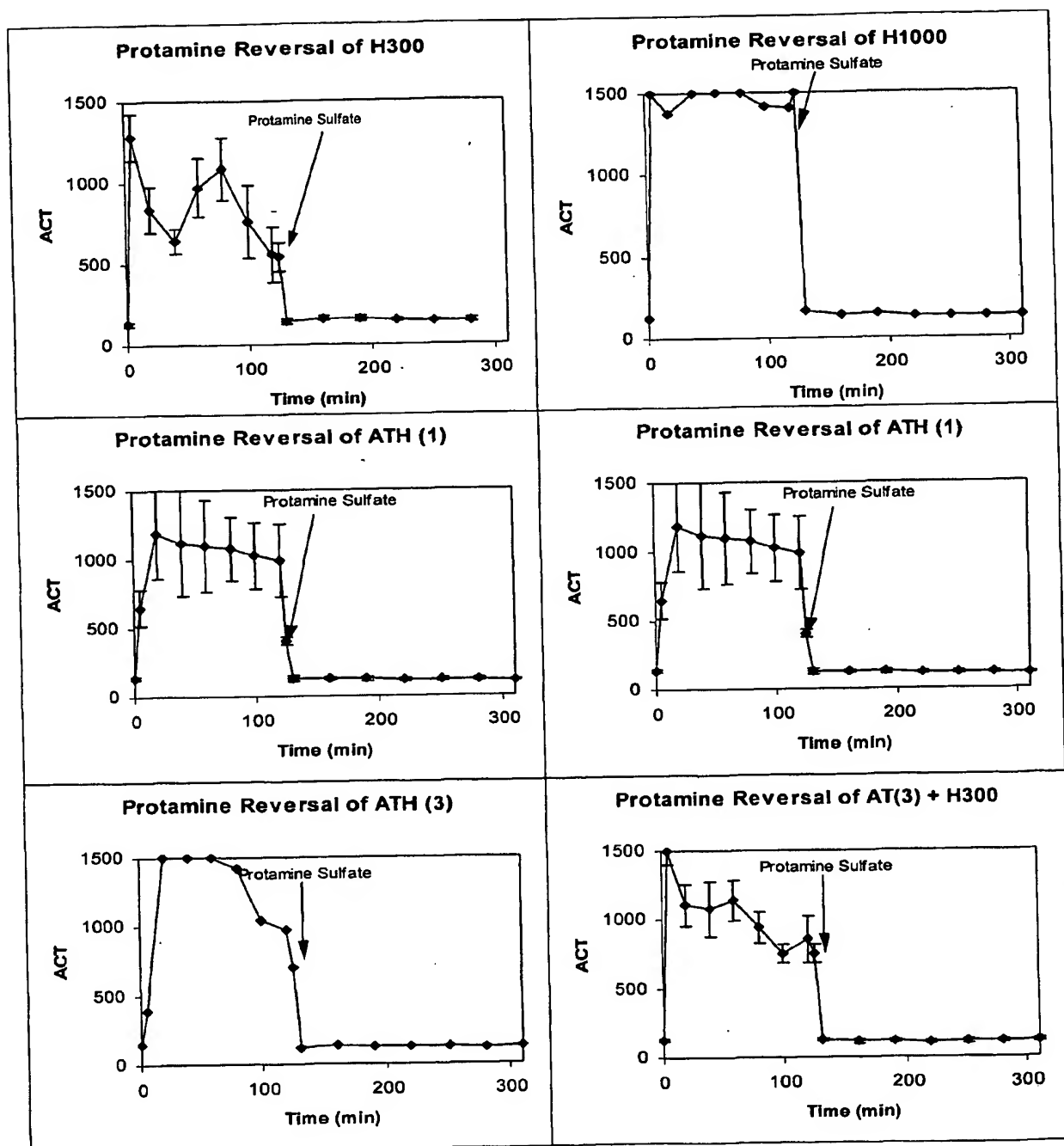


Figure 8

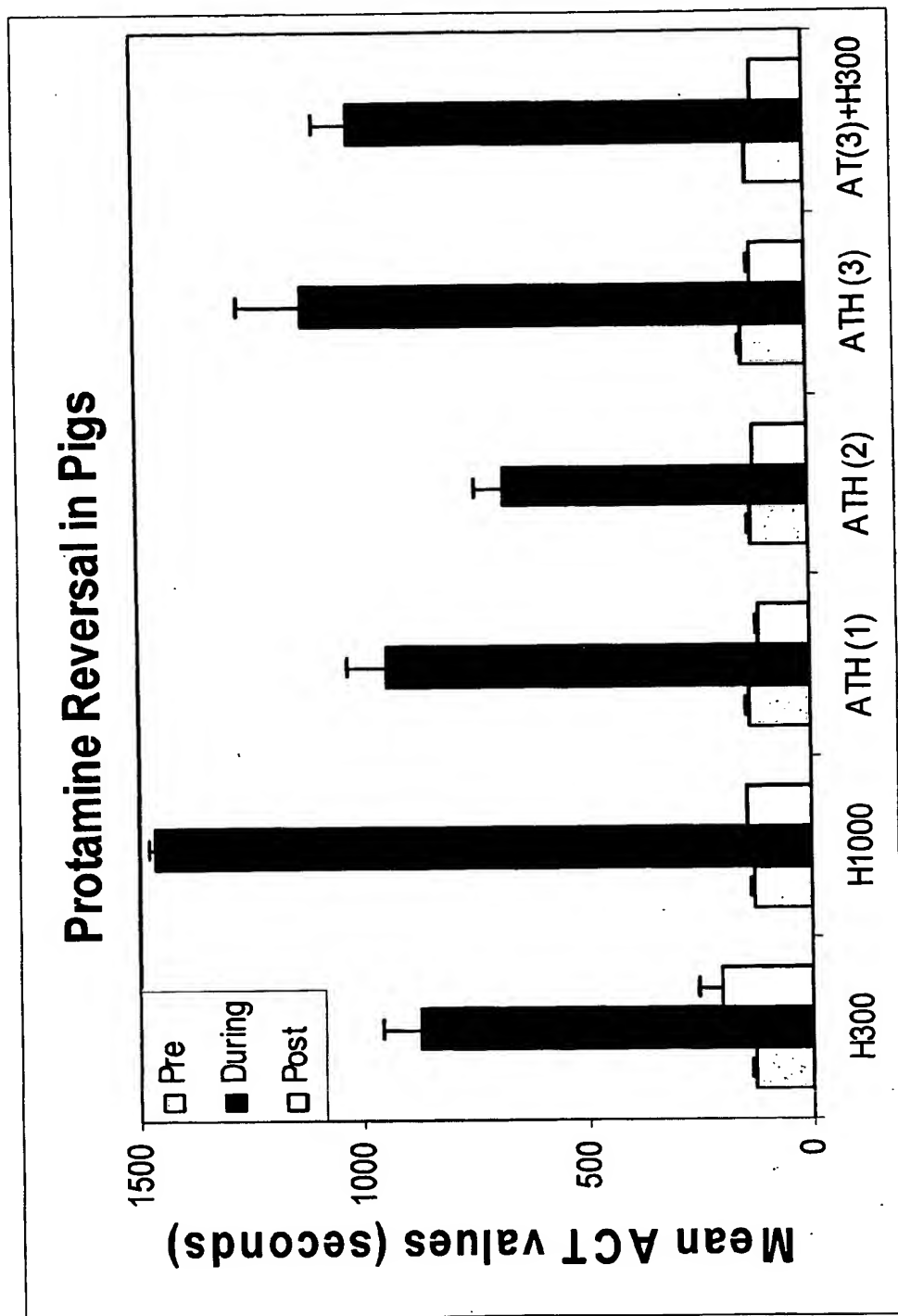


Figure 9

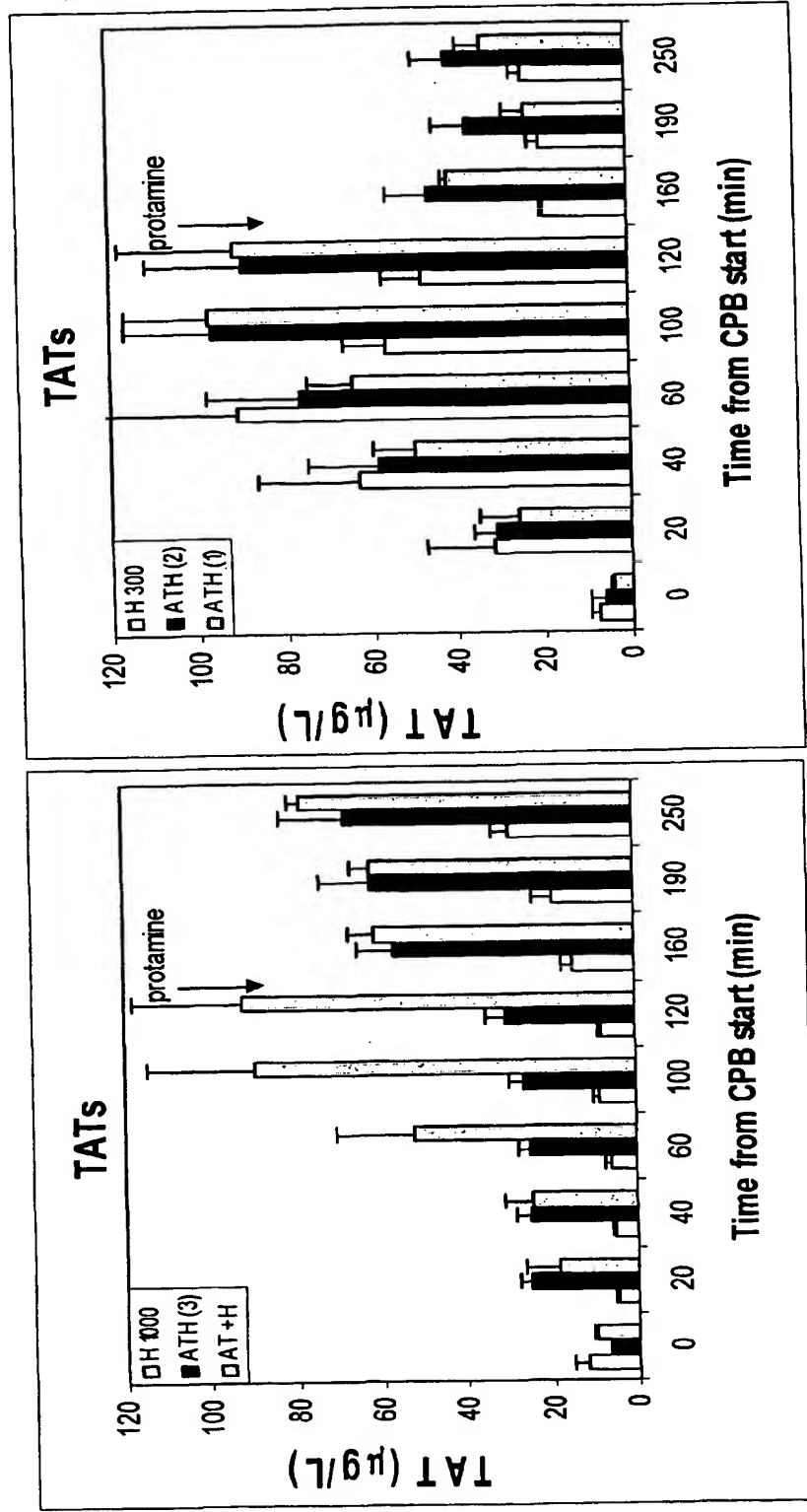
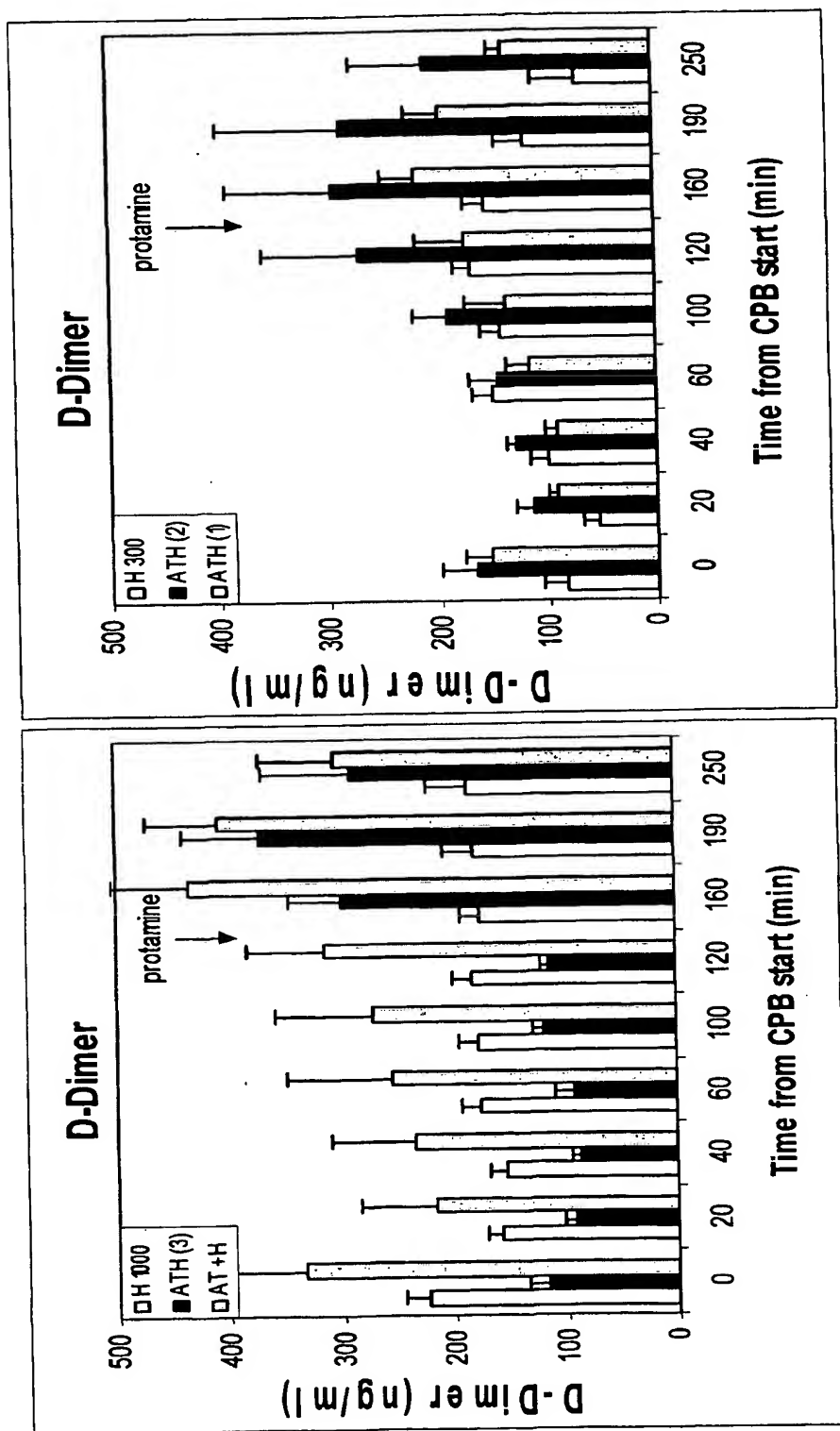


Figure 10



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